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American Chemistry Council *Good Chemistry Makes It Possible*

January 4, 2002

Ms. Christine Todd Whitman
Administrator
U. S. Environmental Protection Agency
P. O. Box 1473
Merrifield, VA 22116

Contain NO CBI

Dear Ms. Whitman:

The American Chemistry Council (Council) makes available to the public and appropriate government agencies final reports of environmental, health, and safety research that it manages. In keeping with this policy, the following final reports that the Council's Brominated Flame Retardant Industry Panel (BFRIP) recently conducted are enclosed:

- Potential for Biotransformation of Radiolabelled Decabromodiphenyl Oxide (DBDPO) in Anaerobic Sediment;
- Effect of Decabromodiphenyl Oxide (DBDPO) on the Survival and Reproduction of the Earthworm, *Eisenia fetida*; and,
- Potential for Biotransformation of Radiolabelled Tetrabromodiphenyl Oxide (TeBDPO) in Anaerobic Sediment.

These reports do not include confidential information.

If you have any questions, please contact Wendy K. Sherman, the BFRIP Manager, at 703/741-5639 or via email [wendy_sherman@americanchemistry.com].

Sincerely yours,

Elizabeth Festa Watson
Managing Director, CHEMSTAR

Enclosures (3)

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POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 439E-104

AUTHORS:
Edward C. Schaefer
R. Scott Flaggs

STUDY INITIATION DATE: January 20, 2000

STUDY COMPLETION DATE: July 25, 2001

AMENDED REPORT DATE: December 20, 2001

Submitted to:

Chemical Manufacturers Association's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

AMENDED

POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

EXECUTIVE SUMMARY

Wildlife International, Ltd. conducted an anaerobic mineralization test to determine the rate and extent of biotransformation and mineralization of commercial product and ^{14}C-labelled decabromodiphenyl oxide, nonvolatile test materials, under anaerobic conditions in a flooded sediment over a 32 week period. The freshwater sediment treatments employed in the mineralization test system consisted of a reference dosed with unlabelled and ^{14}C-labelled d-glucose and two DBDPO treatment groups dosed at nominal concentrations of 5 and 500 mg/kg DBDPO that were used to monitor the production of carbon dioxide ($^{14}CO_2$) and methane ($^{14}CH_4$). Two additional treatment groups were also prepared at 5 and 500 mg/kg. The additional treatment groups were not part of the mineralization (gas collection) system, but were utilized to monitor potential degradation of DBDPO using quantitative analytical methods.

Freshwater sediment samples and accompanying surface water were collected and stored at room temperature in an anaerobic chamber. Twenty test vessels were prepared in the anaerobic chamber one day prior to appropriate amounts of test or reference substance being introduced to their respective test chamber. A resazurin solution prepared using the decanted surface water was added to each vessel after dosing procedures were completed.

The eight test chambers apportioned to the mineralization test system were incubated in a water bath at room temperature (21 to 25°C) throughout the 224 day test period and the production of $^{14}CO_2$ and $^{14}CH_4$ was monitored over time and assayed for radioactivity by liquid scintillation counting (LSC).

Ten gram portions of the day-0 and week-32 dried sediments were extracted. The concentrations of DBDPO in the samples were determined using reversed phase high performance liquid chromatography (HPLC) with UV detection at 220 nm. The extracts were also profiled using a flow-through radioactivity detector (IN/US β-RAM Model 2B).

An average of 95% of the total activity added as radiolabelled glucose was recovered from the sediment in the reference test chambers. Of the recovered activity, 85% was recovered as $^{14}CO_2$ and $^{14}CH_4$ from the mineralization of the radiolabelled glucose and 10% was associated with the sediment. Mineralization of DBDPO was not observed. Less than 1% of the total activity added as decabromodi[U-^{14}C]phenyl ether was recovered as $^{14}CO_2$ and $^{14}CH_4$ indicating that no mineralization of the DBDPO had occurred.

Measured concentrations of DBDPO and radioactivity in the test sediments varied due to the composition of the individual sediment core. Sediments containing greater numbers of gravel/stones had proportionately less sediment and were a source of variability between replicates within and among the test sediments. Radiolabelled components eluting prior to ^{14}C-labelled DBDPO were detected in some of the week-32 5 mg/kg samples. Radiometric detection revealed 1 to 3 peaks in 9 of the 21 samples analyzed. HPLC analysis of a stock solution of the ^{14}C-labelled DBDPO test material also exhibited components eluting prior to the ^{14}C-deca congener using radiometric detection.

Concentrations of DBDPO in the test sediments at the start and conclusion of the study were evaluated using two approaches. In the first approach, seven replicate samples of each test sediment were analyzed by the HPLC-UV procedure. Average measured DBDPO concentrations in the 5 mg/kg sediments on day-0 and week-32 were 6.64 ± 0.70 mg/kg and 6.51 ± 2.15 mg/kg, respectively. Average measured DBDPO concentrations in the 500 mg/kg sediments on day-0 and week-32 were 543 ± 77 mg/kg and 612 ± 158 mg/kg, respectively. Statistical analysis of the data using ANOVA was carried out in order to assess whether the measured concentrations of DBDPO at the start and conclusion of the 32 week test period were significantly different. The F test concluded that the difference between the mean measurements on day-0 and week-32 were not statistically significant (5 mg/kg P = 0.9525; 500 mg/kg P = 0.6555). In the second approach, measured DBDPO concentrations were converted to a DBDPO mass based on the actual dry weight of the sediment and compared to the mass of DBDPO added at test initiation. For the 5 mg/kg sediments, the mean differences between the measured mass and the added mass in day-0 and week-32 samples were 0.123 and 0.127 mg, respectively. For the 500 mg/kg sediments, the mean differences between the measured mass and the added mass in day-0 and week-32 samples were 65.0 and 0.96 mg,

respectively. The difference between the measured mass and mass added was analyzed using a paired t-test. The differences between the DBDPO mass weighed into the test chamber on day-0 and the DBDPO mass calculated using the measured DBDPO concentration at week-32 were also found not to be statistically different (5 mg/kg P = 0.9672; 500 mg/kg P = 0.3764).

Based on the results of this study, DBDPO was neither biotransformed nor mineralized under anaerobic conditions in a flooded sediment over a 32 week period.

AMENDED

STUDY TITLE

Effect of Decabromodiphenyl Oxide on the Survival and Reproduction of the Earthworm, *Eisenia fetida*

DATA REQUIREMENT

U.S. EPA OPPTS Guideline 850.620, OECD Guideline 207, and OECD Proposed Guideline for Earthworm Reproduction Test

AUTHOR

John Aufderheide
ABC Laboratories, Inc.

Timothy Zee Kendall, M.S.
Willard B. Nixon, Ph.D.
Wildlife International, Ltd.

STUDY INITIATION DATE

March 21, 2001

STUDY COMPLETION DATE

December 19, 2001

SPONSOR

American Chemistry Council - BFRIP
1300 Wilson Blvd
Arlington, Virginia 22209

PERFORMING LABORATORY

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

PROJECT IDENTIFICATION

ABC Study No. 46540

Number of Pages: 89

TOXICITY COMPENDIUM

Study Title:	Effect of Decabromodiphenyl Oxide on the Survival and Reproduction of the Earthworm, *Eisenia fetida*
Test Substance:	Decabromodiphenyl Oxide (DBDPO)
Nominal Test Concentrations:	0.00 (Control), 312.6, 650.0, 1,250, 2,500, and 5,000 mg DBDPO/kg of dry soil
Mean Measured Test Concentrations:	< 100 (Control), 320, 668, 1,240, 2,480, and 4,910 mg DBDPO/kg of dry soil
Measured Tissue Concentrations:	All earthworm tissue concentrations of DBDPO, regardless of what test dose concentration of DBDPO was analyzed, were below the level of quantification (< 0.75 micrograms/gram of tissue).
Artificial Soil Characterization:	Sandy Loam (International Textural Class; Hydrometer method)
	Percent Sand: 69
	Percent Silt: 18
	Percent Clay: 13
	% Organic Matter (Carbon): 8.0 (4.7)
	Disturbed Bulk Density: 0.78 gm/cc
	Cation Exchange Capacity: 10.3 (meq./100 g)
Test Species:	*Eisenia fetida,* clitellate adults (347.4 to 587.3 mg of wet weight at test initiation)
Experimental Dates:	Initiation – 27 July 2001 Termination – 21 September 2001
Length of Study:	56 days
Environmental Conditions:	Temperature: 16.8 to 20.7°C
	Soil pH (day 0): 5.86 to 6.07
	Soil pH (day 56): 5.35 to 5.86
	Soil Moisture (day 0): 25.3 to 28.2%
	Soil Moisture (day 56): 36.4 to 45.3%
	Photoperiod: 16-hr light to 8-hr dark
	Light Intensity: 497.3 to 565.2 lux

Conclusions:	28-Day EC_{10} and EC_{50} (survival):	>4,910 mg/kg dry soil
	56-Day EC_{10} and EC_{50} (reproduction):	>4,910 mg/kg dry soil
	NOEC (survival and reproduction):	4,910 mg/kg dry soil

POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED TETRABROMODIPHENYL OXIDE (TeBDPO) IN ANAEROBIC SEDIMENT

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 439E-105

AUTHORS:
Edward C. Schaefer
R. Scott Flaggs

STUDY INITIATION DATE: November 02, 2000

STUDY COMPLETION DATE: December 20, 2001

Submitted to:

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED TETRABROMODIPHENYL OXIDE (TeBDPO) IN ANAEROBIC SEDIMENT

EXECUTIVE SUMMARY

Wildlife International, Ltd. conducted an anaerobic mineralization test to determine the rate and extent of biotransformation and mineralization of nonlabelled and ^{14}C-labelled 2,2',4,4'-tetrabromodiphenyl oxide (TeBDPO) (CAS 5436-43-1) under anaerobic conditions in flooded sediment over a 32-week period. The freshwater sediment treatments employed in the mineralization test system consisted of a reference sediment dosed with unlabelled and ^{14}C-labelled d-glucose and two TeBDPO treatment groups dosed at nominal concentrations of 5 and 500 mg/kg TeBDPO that were used to monitor the production of carbon dioxide ($^{14}CO_2$) and methane ($^{14}CH_4$). Two additional treatment groups were also prepared at nominal concentrations 5 and 500 mg/kg. The additional treatment groups were not part of the mineralization (gas collection) system, but were utilized to monitor potential degradation of TeBDPO using quantitative analytical methods.

Freshwater sediment samples and accompanying surface water were collected and stored at room temperature in an anaerobic chamber. Twenty test vessels were prepared in the anaerobic chamber one day prior to appropriate amounts of test or reference substance being introduced to their respective test chamber. Surface water containing the redox indicator resazurin was added to each vessel after dosing procedures were completed.

The eight test chambers apportioned to the mineralization test system were incubated in a water bath at room temperature (20 to 24 °C) throughout the 224 day test period and the production of $^{14}CO_2$ and $^{14}CH_4$ was monitored over time and assayed for radioactivity by liquid scintillation counting (LSC).

Five gram portions of the day-0 and week-32 dried sediments were extracted. The concentrations of TeBDPO in the samples were determined using reversed phase high performance liquid chromatography (HPLC) with UV detection at 236 nm. The extracts were also profiled using a flow-through radioactivity detector (IN/US β-RAM Model 2B).

An average of 101% of the total activity added as radiolabelled glucose was recovered from the sediment in the reference test chambers. Of the recovered activity, 81% was recovered as $^{14}CO_2$ and $^{14}CH_4$ from the mineralization of the radiolabelled glucose and 20% was associated with the sediment. Mineralization of TeBDPO was not observed. Less than 1% of the total activity added as tetrabromodi[U-^{14}C]phenyl ether was recovered as $^{14}CO_2$ and $^{14}CH_4$ indicating that no mineralization of the TeBDPO had occurred.

Measured concentrations of TeBDPO and radioactivity in the test sediments varied due to the composition of the individual sediment core. Sediments containing greater numbers of gravel/stones had proportionately less sediment and were a source of variability between replicates within and among the test sediments. Radiolabelled components eluting prior to ^{14}C-labelled TeBDPO were detected in a number of the week-32 samples. Radiometric detection revealed 1 to 3 significant peaks in 26 of the 42 samples analyzed. At least 1 significant peak was observed in all of the 500 mg/kg week-32 sample radiochromatograms. HPLC analysis of a stock solution of the ^{14}C-labelled TeBDPO test material also exhibited components eluting prior to the ^{14}C-tetra congener using radiometric detection.

Concentrations of TeBDPO in the test sediments at the start and conclusion of the study were evaluated using two approaches. In the first approach, seven replicate samples of each test sediment were analyzed by the HPLC-UV procedure. Average measured TeBDPO concentrations in the 5 mg/kg sediments on day-0 and week-32 were 6.49 ± 1.35 mg/kg and 7.53 ± 1.67 mg/kg, respectively. Average measured TeBDPO concentrations in the 500 mg/kg sediments on day-0 and week-32 were 832 ± 69 mg/kg and 771 ± 127 mg/kg, respectively. Statistical analysis of the data using ANOVA was carried out in order to assess whether the measured concentrations of TeBDPO at the start and conclusion of the 32 week test period were significantly different. The F test concluded that the difference between the mean measurements on day-0 and week-32 were not statistically significant (5 mg/kg $P = 0.4389$; 500 mg/kg $P = 0.4812$). In the second approach, measured TeBDPO concentrations were converted to a TeBDPO mass based on the actual dry weight of the sediment and compared to the mass of TeBDPO added at test initiation. For the 5 mg/kg sediments, the mean differences between the measured mass and the added mass in day-0 and week-32 samples were 0.059 and 0.276 mg, respectively. For the 500 mg/kg sediments, the mean differences between the measured

mass and the added mass in day-0 and week-32 samples were 59.9 and 46.5 mg, respectively. The difference between the measured mass and mass added was analyzed using a paired t-test. The differences between the TeBDPO mass weighed into the test chamber on day-0 and the TeBDPO mass calculated using the measured TeBDPO concentration at week-32 were also found not to be statistically different (5 mg/kg P = 0.5366; 500 mg/kg P = 0.6862).

Based on the results of this study, TeBDPO was not mineralized under anaerobic conditions in a flooded sediment over a 32 week period. Evidence of biotransformation was observed primarily in the 500 mg/kg treatments.